# Using metagenomics to investigate the impact of hospital stay and the ARK intervention on the human gut resistome

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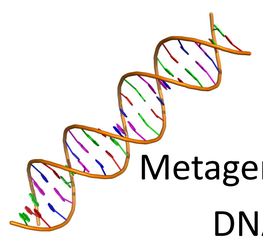
Antimicrobials are vital for modern medicine. Antimicrobial use selects for antimicrobial resistant bacteria, particularly among the gut microflora. Minimising antimicrobial resistance (AMR) selection by avoiding unnecessary antibiotic use helps combat AMR. Metagenomic analyses have the potential to provide accurate detection and quantification of AMR genes within an individual's gut microbiome (gut 'resistome'), allowing the impact of different types of antibiotic exposures to be evaluated and guide interventions to reduce AMR.

We have developed a short-read sequencing approach to characterise the gut resistome and piloted this in two clinical sample sets.

## Method

faecal sample

DNA extraction →



Metagenomic DNA

DNA sequencing →

Sequence to a depth of ~10 million reads

### Bioinformatics Analysis

- Remove sequencing adaptors and low-quality / human reads.
- Match the cleaned sequencing reads to known AMR genes in CARD database (DOI: 10.1093/nar/gkw1004) using the programme ARIBA (DOI: 10.1099/mgen.0.000131)
  - this determines what AMR genes are present in each sample.

## ACLOD Cohort (Journal of Infection (2017) 75, 20-25)

- Participants for this study were patients admitted to elderly medicine wards at three UK hospitals; criteria included being over 18 and being negative for *C. difficile* infection.
- After consenting, participants were asked to provide weekly stool samples during their hospital stay.
- From those collected, we sequenced two samples from 25 participants at two of the hospitals (n=50).

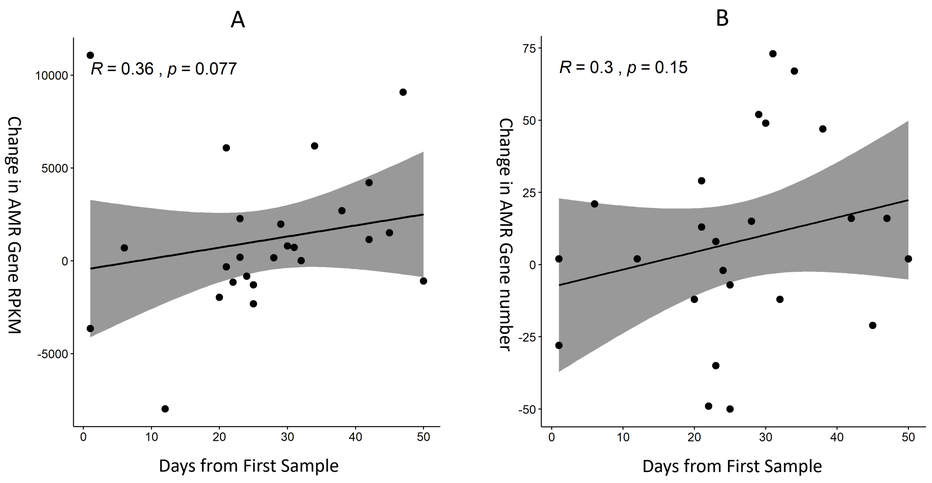



**Figure 1**: changes in AMR gene reads/kb/million total reads (RPKM, A) and AMR gene number (B) between the first and second samples from each patient, with Spearman's correlation (n=25).

Fig. 1 shows changes in the number of AMR gene reads/kb/million total reads (RPKM, A) and AMR gene number (B)

- There is no significant association between length of hospital stay and AMR gene carriage.

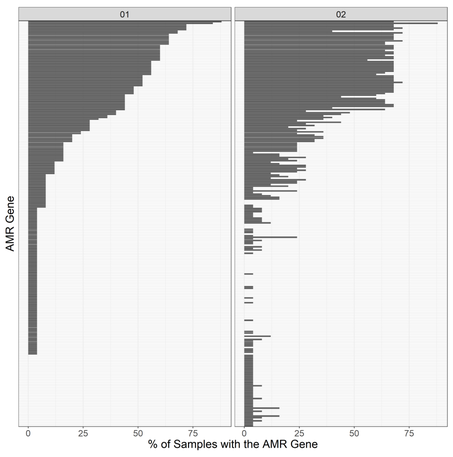



**Figure 2**: the frequency of AMR genes in the first (01) and second (02) samples (n=25 for each time point).

Fig. 2 shows which AMR genes were most frequently seen in the first sample (01) and second sample (02)

- tetW (88%) is the most frequent in the first sample, while dfrF (88%) is the most frequent in the second.
- There seems to be more AMR genes present in the second sample compared to the first.

## ARK Cohort

- Antibiotic Review Kit (ARK) is a behavioural intervention which aims to aid healthcare workers to reduce unnecessary antibiotic use in hospitals.
- The impact of the ARK intervention on the human gut resistome is being analysed by utilising discards from diarrhoeal samples which were sent to the microbiology lab for *C. difficile* testing at a UK hospital (n=83), collected before and after the ARK intervention was implemented there.

Fig. 3 shows how the AMR gene reads/kb/million total reads (RPKM) changes over the sample collection period.

- There seems to be an increase in AMR gene RPKM over time.
- Month 5, one month after ARK implementation, there is a drop in AMR gene RPKM.

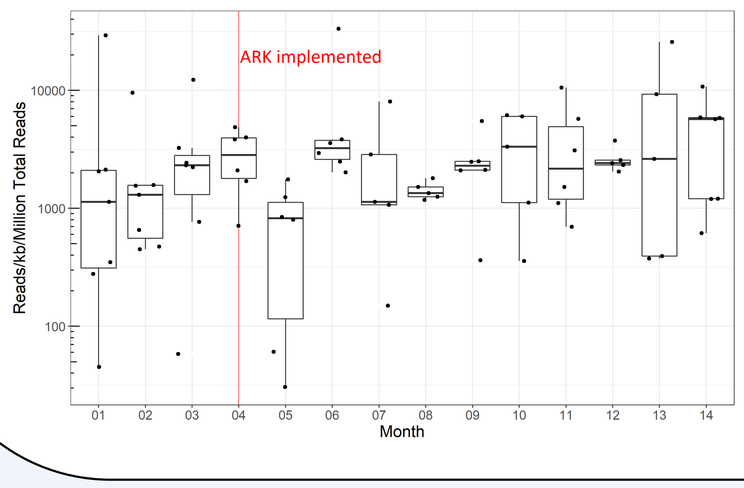



**Figure 3**: changes seen in AMR gene reads/kb/million total reads (RPKM) before and after the implementation of the ARK intervention at a UK hospital (n=5 to 7 for each month).

Fig. 4 shows how the frequency of selected AMR genes change over time.

- There are variations in how the frequency of AMR genes changes over the course of sample collection (14 months).
- However, small sample numbers at each time point (n=5 to 7) makes it difficult to draw meaningful conclusions about the changes.

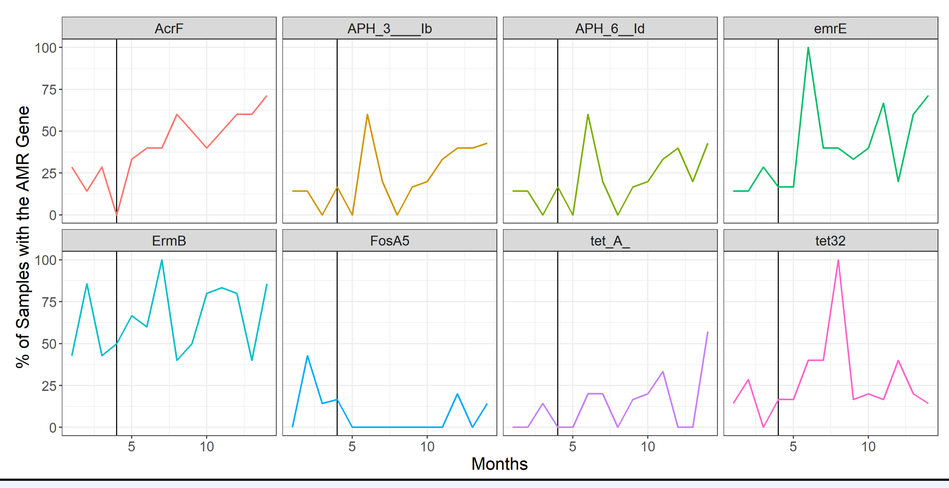



**Figure 4**: changes in the frequency of selected AMR genes over the sample collection period (14 months, n=5 to 7 for each month). Black lines indicate when the ARK intervention was implemented.

## Conclusions

- Direct, deep sequencing can be used to detect AMR genes in faecal samples.
- Longer hospital stays do not show a statistically significant difference in AMR gene RPKM/number, but more data may reveal an effect.
- AMR gene RPKM seems to increase in the ARK cohort, but is difficult to conclude due to the low sample numbers and high variability.

## Further work

- Apply a regression model to the ACLOD data, as an alternative method to look at the changes in AMR gene carriage over hospital stay.
- More samples from this ARK cohort, as well as those from two other "ARK participating hospitals", will be analysed to add to the existing dataset.
- Perform an interrupted time series analysis to see if the ARK intervention slows down the increase in AMR genes seen in this cohort.

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